Fruit-mate™ for RNA Purification (Cat.# 9192)

Application: Improved RNA Isolation by Pretreatment with Fruit-mate™ for RNA Purification

Fruit-mate™ for RNA Purification (Cat.# 9192) is a pretreatment reagent used prior to isolating total RNA from plant tissue samples with high polysaccharide or polyphenol content (e.g., roots, fruits, seeds, etc.). The Fruit-mate reagent contains a non-ionic polymer that binds to these substances and allows them to be removed by centrifugation.

Methods:

Pre-treatment with Fruit-mate reagent and RNA isolation using NucleoSpin RNA Plant (Clontech Cat.# 740949.50):

1. At least 100 mg of plant tissue was placed in a mortar and pulverized under liquid nitrogen using a pestle.
2. 50 mg of the pulverized plant tissue was transferred into a cold (liquid nitrogen) 1.5 mL RNase-free tube. 500 µl of Fruit-mate reagent was added and mixed well.
3. The mixture was immediately centrifuged at 12,000 × *g* at 4°C for 5 minutes. The supernatant was carefully removed and placed in a new 1.5 mL RNase-free tube (100 µl per tube).
4. For each 100 µl of supernatant, 350 µl of buffer RA1 (+DTT) or buffer RAP (+DTT) was added and the tubes were vortexed well. RA1 and RAP lysis buffers are provided in the NucleoSpin RNA Plant kit.
5. The mixture was applied to a NucleoSpin Filter (violet ring), placed in a 2 mL tube, and centrifuged at 11,000 × *g* at room temperature for 1 minute.
6. The filter was removed. If a pellet was formed in the 2 mL tube, the supernatant was carefully transferred to another tube.
   350 µl of 70% ethanol was added to the flowthrough, and the solution was mixed thoroughly by pipetting. The mix ture was applied to the NucleoSpin RNA Plant Column (light blue ring), and the column was centrifuged at 11,000 × *g* at room temperature for 30 seconds.
7. The NucleoSpin RNA Plant Column (light blue ring) was transferred to a new 2 mL tube. 350 µl of MDB (supplied in the NucleoSpin RNA Plant kit) was applied, and the column was centrifuged for 1 minute at 11,000 × *g* at room temperature. After, the flowthrough was removed.
8. 95 µl of DNase solution (provided in the NucleoSpin RNA Plant kit) prepared as described in the NucleoSpin RNA Plant User Manual was applied to the center of the NucleoSpin RNA Plant Column (light blue ring) membrane. The reaction was allowed to proceed for 15 minutes at room temperature (20–25°C).
9. 200 µl of buffer RA2 was applied, and the column was centrifuged at 11,000 × *g* at room temperature for 30 seconds.
10. The NucleoSpin RNA Plant Column (light blue ring) was transferred to a new 2 mL tube. 700 µl* of buffer RA3 was applied, and the column was centrifuged for at 11,000 × g at room temperature for 30 seconds.

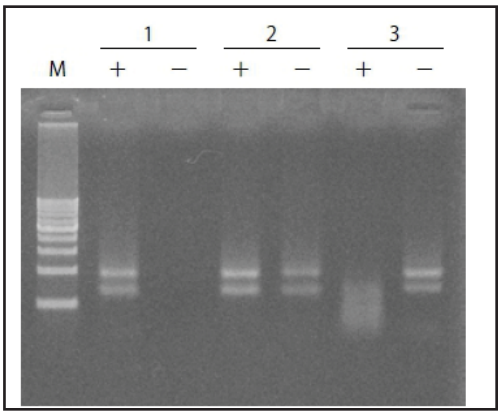
    * The NucleoSpin RNA Plant protocol recommends 600 µl, but 700 µl is preferable for this application.

11. The flowthrough was removed and 250 µl of buffer RA3 was added to the column. The column was centrifuged at 11,000 × *g* at room temperature for 2 minutes.
12. The NucleoSpin RNA Plant Column (light blue ring) was transferred to a new 1.5 mL tube. 60 µl of RNase-free H_2O was applied, and the column was centrifuged at 11,000 × *g* at room temperature for 1 minute.
13. If the recovered RNA was not used immediately for analysis or other downstream applications, it was stored between −20°C and −80°C.

Application: Improved RNA Isolation by Pretreatment with Fruit-mate™ for RNA Purification (continued)

Results:

Example 1: Total RNA was extracted from various plant tissues using buffer RA1 following the methods described above. The extraction efficiency with or without pretreatment with Fruit-mate reagent was compared by gel electrophoresis.

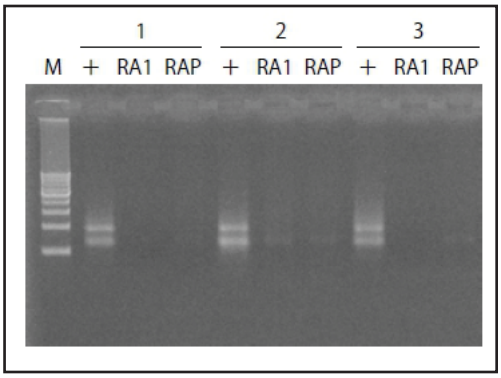


Lane	Sample
M	1 kb ladder
1	Banana (Fruit flesh)
2	Mini (cherry) tomato (Fruit pericarp)
3	*Hypsizygus tessellatus*, shimeji mushroom (cap and stem)*

*No beneficial effects of treatment with Fruit-mate reagent were observed.

Figure 1. Analysis of total RNA by gel electrophoresis. Equal amounts of total RNA were loaded. RNA was extracted using NucleoSpin RNA Plant with (+) or without (−) pre-treatment with Fruit-mate reagent.

Example 2: Total DNA was extracted from various plant tissues using buffer RA1 or buffer RAP according to the methods above. The extraction efficiency with or without pre-treatment with Fruit-mate reagent was compared by gel electrophoresis.



Lane	Sample
M	1 kb ladder
1	Potato (tuber)
2	Chrysanthemum (leaves)
3	Rose (leaves)

Figure 2. Analysis of total RNA by gel electrophoresis. Equal amounts of total RNA were loaded. RNA was extracted using buffer RA1 or buffer RAP alone (RA1 and RAP, respectively) or after pre-treatment with Fruit-mate reagent and extraction with buffer RA1 (+).

Conclusions:

RNA isolation from many plant samples is difficult using convention methods because of the high polysaccharide or polyphenol content of these tissues. Pre-treatment with Fruit-mate reagent improved the yield and quality of RNA isolated from various plant samples, including banana (fruit), mini tomato (fruit), potato (tuber), chrysanthemum (leaves), and rose (leaves).